AD_____

Award Number: W81XWH-12-1-0213

TITLE: Targeting G-Protein Signaling for the Therapeutics of Prostate Tumor Bone Metastases and the Associated Chronic Bone Pain

PRINCIPAL INVESTIGATOR: Songhai Chen

CONTRACTING ORGANIZATION: University of Iowa, Iowa City, IA 52242

REPORT DATE: July 2014

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
July 2014	Annual	1 July 2013-30 June 2014

4. TITLE AND SUBTITLE
Targeting G-Protein Signaling for the Therapeutics of Prostate Tumor Bone Metastases and the Associated Chronic Bone Pain

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-12-1-0213

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Songhai Chen (PI)
Yuanchao Ye (Postdoctoral fellow)

E-Mail: Songhai-chen@uiowa.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
The University of Iowa, Iowa City, IA 52242

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
Bone tumor metastasis is the major cause of mortality and morbidity in advanced prostate cancer patients. These patients frequently suffer from moderate to severe chronic bone pain. However, the current treatments for these patients are ineffective and non-curative, because metastatic tumors are resistant to most of the current anti-cancer treatments, and the currently used pain therapeutic medications or analgesics often do not provide effective relief from pain due to the development of tolerance upon chronic use, and also have serious side effects. Thus, it is imperative to develop novel approaches that can both effectively block tumor growth in bones and relieve the associated bone cancer pain. This proposal aims to define the key role of the Gβγ subunits of heterotrimeric G proteins in the development of prostate tumor bone metastasis and the associated bone pain. Our studies by far have demonstrated that Gβγ signaling plays a key role in mediating proliferation of several prostate cancer cell lines, including LNCaP, PC3, DU145 and 22Rv1. Moreover, we show that activation of G protein-coupled receptor does not cause transactivation of androgen receptors. Rather, these receptors may stimulate prostate cancer cell growth and migration through several signaling pathways, including PI3K/AKT, ERK and calcium signaling that are activated downstream of Gβγ .

15. SUBJECT TERMS
Cancer Pain, Prostate Cancer Bone Metastasis, heterotrimeric G protein βγ subunits, G protein-coupled receptors, signal tranduction

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	6	19b. TELEPHONE NUMBER *(include area code)*

Table of Contents

1. Introduction:

Bone metastasis is one of the most common and severe complications in advanced prostate cancer. It is the major cause of mortality and morbidities, due to the development of bone pain, hypercalcemia, fractures, spinal cord compression and consequent paralysis. The current regimen for these patients is largely palliative and non-curative, because metastatic tumors are resistant to most of the current anti-cancer treatments. Thus, it is imperative to develop novel therapeutic approaches for the treatment of advance prostate cancer. This proposal aims to define the key role of the Gβγ subunits of heterotrimeric G proteins in the development of prostate tumor bone metastasis and the associated bone pain, as well as determine the potential therapeutic efficacy of targeting Gβγ with small molecule inhibitors in preclinical models of bone-metastasized prostate cancer. G proteins mediate the function of a large group of cell surface receptor proteins called G protein-coupled receptors (GPCRs). Comparative experimental and clinical evidence has indicated that excessive activation of the GPCR systems due to overexpression of the receptors and their ligands in prostate tumor cells or their surrounding cells contributes to the metastatic spread of tumor cells to bones, their subsequent growth there and the consequent bone destruction. Moreover, continue activation of GPCRs in the sensory nerve fibers adjacent to bones results in increased activity/expression of key pain-sensing receptor channels, such as TRPV1, such that the channels are constitutively activated, leading to the sensation of chronic pain without any overt stimulation. Thus, the GPCR system represents an attractive target for the therapeutics of bone tumor metastasis and the associated bone pain. However, the involvement of several dozen GPCRs and their ligands in tumor progress has presented a significant hurdle for the progression of such approach. Given that G proteins function downstream of GPCRs, we propose to thoroughly investigate the role of Gβγ in mediating signals from multiple GPCRs to promote prostate tumor growth and metastasis and for the associated bone cancer pain, using both *in vitro* cell culture and *in vivo* preclinical model of prostate tumor metastasis. Considering the recent discovery of a series of small molecule inhibitors of Gβγ that have been successfully used in the treatment of several pathologies in the preclinical mouse models of heart failure, inflammation, opioid receptor-dependent analgesia and morphine-induced antinociceptive tolerance and dependence, without causing overt side effects, results from our proposed studies have the potential to uncover a novel and efficacious approach for the development of new mechanism-based therapies to improve the outcome of advanced prostate cancer patients, including the men in the military services who are suffering from this disease.

2. Keywords: Prostate Cancer Bone Metastasis, Bone Cancer Pain, Heterotrimeric G protein betagamma subunits, G protein coupled receptors (GPCRs), TRPV1, Nociceptor Sensitization

3. Overall project summary: Summarized below are the accomplishments from research work performed in the 2nd yr of this project in direct alignment with the Statement of Work (SOW) schedule.

Milestone-1: Determine the role of Gβγ signaling in mediating prostate tumor cell growth, migration and invasion *in vitro*, as well as mediating GPCR-regulated TRPV1 channel function in cultured mouse sensory neurons (Aim 1).

Major Goal/Objective 1: Determine the role of Gβγ signaling in regulating prostate tumor cell growth, migration and invasion (months 1-12).

1e. Determine the role of Gβγ signaling in the transactivation of androgen receptor (months 9-12).
Accomplishments: To determine if Gβγ regulates prostate tumor cell growth and migration in part via regulating androgen receptor activity, we monitored androgen receptor transcriptional activity in LnCaP and 22v1. These studies were delayed in the first year of the study because it has taken us a while to obtain the luciferase reporter genes for androgen receptor. Using a reporter gene containing the PSA promotor (-4841 to +12) fused to luciferase, we found in LnCaP and 22Rv1 cells that activation of androgen receptors by dihydrotestosterone resulted in a significant increase in luciferase activity (Fig. 1). Activation of several G protein coupled receptors by SDF1a, LPA and bradykinin to initiate Gβγ signaling, either alone or in combination with dihydrotestosterone, did not cause a significant change in luciferase activity. These findings suggest that activation of Gβγ signaling does not cause transactivation of androgen receptors.

Major Goal/Objective 2: Determine the role of Gβγ signaling in mediating GPCR-stimulated upregulation of TRPV1 expression/function in cultured mouse DRG sensory neurons. (months 12-18).

1a. Generating adenovirus encoding EGFP, Gβ1γ2 and Gαt for modulating Gβγ signaling in cultured DRG sensory neurons. (months 12-15)
Accomplishments: We have generated and purified the adenovirus. We then tested the efficacy of the virus to express the target proteins in PC3 cells. While transducing PC3 cells with the virus led to a high level of protein expression (Fig. 2A), unexpectedly, we found AKT but not ERKs was activated in cells transduced with control virus expressing GFP in a viral dose-dependent manner (Fig. 2B). Since AKT activation will likely activate TRPV1 channels in DRG neurons, we cannot further assess the effect of Gβ1γ2 and Gαt expression on TRPV1 activity. We are currently exploring the possibility of expressing Gβ1γ2 and Gαt using lentivirus in DRG neurons.

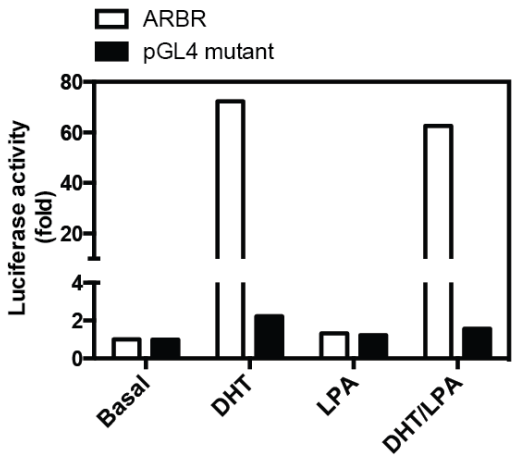

Fig 1. Activation of androgen receptor transcriptional activity by GPCRs and androgen receptors. LNCaP cells transfected with a luciferase reporter gene (ARBR) and its negative control (pGL4 mutant) were treated with LPA (10 μM), dihydrotestosterone (DHT; 0.1 μM) or LPA plus DHT (DHT/LPA) for 24 hr. Luciferase activity was measured using dual luciferase assay kits.

1b. Determine the effect of Gβγ signaling on TRPVI channel activity and nociceptor firing. (months 15-17)
This study was postponed because of the issues described above

1c. Determine the effect of Gβγ signaling on TRPVI protein expression by Western blotting and immunostaining analyses (months 17-18). This study was postponed because of the issues described above

Milestone-2: Determine the efficacy of blocking Gβγ signaling on prostate tumor bone metastasis, and chronic bone pain in a xenograft mouse model (Aim 2). (months 12-36).

Major Goal/Objective 1: Development of *scid* mouse xenografts of human prostate cancer cells and characterization/assessment of the effects of blocking Gβγ signaling on the formation of bone tumor metastases and bone-related pain behavior in these mice. (months 18-24)

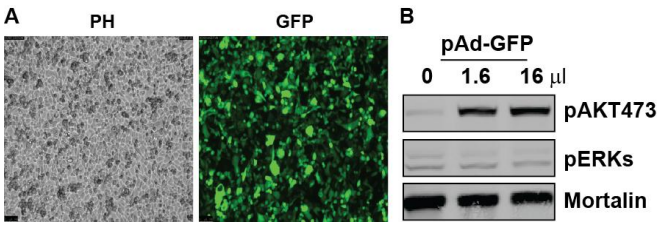

Fig 2. Activation of AKT in PC3 cells transduced by adenovirus. PC3 cells were transduced by different amount of adenovirus encoding GFP (pAD-GFP). Phosphorylation of AKT and ERKs was determined in cell lysates 72 hr post-transfection. A, phase-contrast and GFP fluorescence imaging of cells transduced with pAD-GFP. B, Western blotting analysis of AKT and ERK phosphorylation. Mortalin was used as a loading control.

1a. Assessing the effects of blocking Gβγ signaling on bone tumor metastases and survival of *scid* mice with prostate cancer cell xenografts (months 18-24). We injected PC3 cells expressing inducible EGFP or the Gβγ scavenger Gαt via intracardiac injection into male C57BL/6-Rag1-scid mice to generate bone metastasis models of prostate cancer. To our surprise, these mice did not develop any detectable tumors over two months post-injection (Fig. 3). Similarly, s,c injection of PC3 into these mice did not result in any tumor formation, suggesting that these mice may have residual immunity that inhibits human tumor formation. Because of this, we decided to switch to nude mice or C.B.17 *scid* mice for our studies. We have recently amended the protocol and obtained the approval from our institute and USAMRMC. We expect to generate the xenograft mouse models of prostate cancer in these mice in 3-5 months.

1b. Assessing the effects of blocking Gβγ signaling on bone-related pain behavior. (months 18-24).). This study was delayed because of the issues described above

1c. Through evaluation of metastatic bone tumor-induced chronic pain behavior in these mouse xenografts by statistical analyses (months 22-24). This study was delayed because of the issues described above

4. Key research accomplishments:

- In our second year of this study, we further determined the mechanisms by which Gβγ signaling promotes prostate cancer cell growth and migration. We showed Gβγ signaling does not trans-activate androgen receptors, indicating that Gβγ likely functions, independent of androgen receptors, through its downstream signaling pathways such as ERK and AKT to stimulate prostate cancer cell proliferation and migration. This suggests that concurrently blocking Gβγ signaling and androgen receptors may have synergistic effects on inhibiting prostate cancer cell growth and migration. These findings thus support our hypothesis that targeting Gβγ signaling may represent a novel therapeutic approach for the treatment of prostate cancer.
- We begin to establish in vivo mouse models of prostate bone metastasis.

5. Conclusion:

In conclusion, our results from the second year of this study have led us to show that Gβγ signaling functions independent of androgen receptors in promoting prostate tumor cell growth and migration. These findings thus provide important support for targeting Gβγ signaling as an adjunct approach for prostate cancer treatment. Currently, we are continuing with studies to determine the role of Gβγ signaling in regulating the expression/function of the pain-sensing channel TRPV1, and the function of Gβγ signaling in promoting prostate tumor progression and bone cancer pain in vivo.

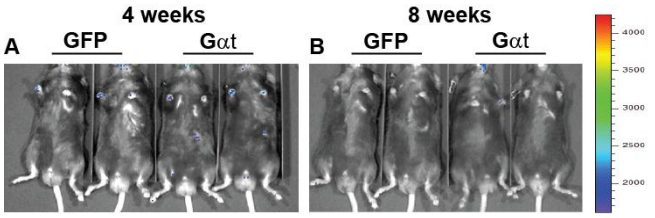

Fig 3. Monitoring PC3 cell growth in C57BL/6-Rag1-*scid* mice by bioluminescence imaging. PC3 cells expressing inducible EGFP or the Gβγ scavenger Gαt were injected into male C57BL/6-Rag1-*scid* mice via intracardiac injection. PC3 cell growth was monitored by bioluminescence imaging at the indicated time post-injection.

6. Publications, Abstracts, and Presentations

Scientific presentations: My postdoc and I attended the 2014 AACR meeting, and I was also invited by Georgia Reagents University to give a seminal on the function of Gβγ signaling in promoting tumor progression.

7. Inventions, Patents and Licenses: no

8. Reportable Outcomes: n/a

9. Other Achievements: n/a

10. References: n/a

Appendices: n/a